therapeutic and diagnostic agents, across, for example, interior cell membranes, such as those of endosomal vesicles within cells. The compositions include (1) a pH-sensitive polymer which does not disrupt cell membranes at physiological pH but which disrupts the endosomal membrane at the pH range inside the endosomes in combination with (2) a second component which further enhances disruption and/or delivery of a therapeutic or diagnostic agent. The second component is conjugated or incorporated into the first polymer, and the composition optionally can be provided in a carrier such as nanoparticles, microparticles, and liposomes.

As discussed with the examiner at the very helpful interview on April 5, 2001, the type of membranes applicants are referring to are membranes in cells, cell components such as endosomal membranes, and liposomes or lipid vesicles. As discussed below with regard to the prior art, applicants are not talking about membranes such as those found in drug delivery devices. As observed by the examiner, and drawn by Dr. Stayton on the enclosed diagram in response to the examiner's comments, the polymer conjugates which have been developed are able to alter transport through these cell membranes by changing from a hydrophilic or hydrophobic to a **more hydrophobic more lipophilic** state in response to a stimulus, such as a change in pH. The claims have therefore been amended to more clearly define these points. Support for these amendments are found in the application at page 9, lines 19-21, and at pages 10-17. Claim 28 has also been amended to specifically refer to polymers described in the specification at page 14, last line, and page 6, line 26.

II. Rejections Under 35 U.S.C. § 112

Claims 1, 5, 7-13, 15 and 17-32 were rejected under 35 U.S.C. § 112, second paragraph,

as indefinite. The rejection is respectfully traversed if applied to the claims as amended.

As discussed above, the term "membrane" has been replaced with the more explicit terms " cell membranes", "cell component membranes", and "liposomes or lipid vesicles".

It is not clear what the objection is with respect to claim 5. Claim 5 refers to claim 1 where the conjugate is coupled to a diagnostic or therapeutic agent, which further includes a pharmaceutically acceptable carrier. Claim 1 includes the alternative embodiment where the conjugate is coupled to a carrier rather than to a therapeutic or diagnostic agent, such as a microsphere to be delivered or an affinity agent. The claim has been amended to more clearly define this relationship.

Claim 7 has been amended to recite "blend thereof". Claim 15 was cancelled and newly added as claim 33. This obviates the concern that the claim depends from higher number claims.

The term "solvent composition" in claim 22 has been amended to refer to a change in solvent to alter solubility of the composition. This is described at page 17, line 6 (different solubility as reported by...any of several references).

The objection to claim 9 was discussed at the interview. The compounds which are recited decrease lysosomal degradation of a material in the lysosome either by decreasing production of acid within the lysosome, or by decreasing the acidity of the lysosome directly. In the former case, this may be by inhibition of enzymes involved in acid production; in the latter case, this may be by buffering or neutralizing the acid which is produced.

III. Rejections Under 35 U.S.C. § 102

Claims 1, 5, 7, 9, 15, 18, 19, 21, 22 and 28-29 were rejected under 35 U.S.C. § 102(a) as

anticipated by U.S. Patent No. 5,609,590 to Herbig et al. ("Herbig"). Claims 1, 5, 7-13, 15, and 18-32 were rejected under 35 U.S.C. § 102(a) as anticipated by PCT WO 97/09068 by Hoffman and Stayton ("Hoffman"). Claims 1, 5, 7, 9, 10, 15, 18-25 and 32 were rejected under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,807,306 to Shapland et al. ("Shapland"). Claims 1, 3, 5, 8-13, 15, 17-19, 21, 22, 27 and 30 were rejected under 35 U.S.C. § 102(e) as anticipated by, or under § 103 as obvious over, U.S. Patent No. 5,753,263 to Lishko et al. ("Lishko"). The rejections are respectfully traversed if applied to the amended claims.

Herbig

Herbig discloses osmotic bursting devices for dispensing a drug in an aqueous environment (abstract). The devices utilize a pH sensitive material coating a drug capsule, such that the pH sensitive material is semipermeable to water and causes hydrostatic pressure within the capsule to build and eventually burst the capsule, or the pH sensitive material is used to hold together two capsule portions until it dissolves or disintigrates in contact with an environment having a particular pH to release the capsule contents (col. 6, lines 40-58). There is no disclosure or suggestion in Herbig, however, of a pH-sensitive polymer which alters transport through a cell or cell component membrane at a pH between about 5 and 6.5 by becoming more hydrophobic and more lipophilic and which is conjugated to or has incorporated therein a second polymeric or monomeric unit which bonds to a carrier or a therapeutic or diagnostic agent.

Mitragotri

Mitragotri discloses the transdermal transport of drugs using low frequency ultrasound, chemical modifiers of permeability and/or cavitation, iontophoresis and/or electroporation,

pressure and/or vacuum and magnetic force fields (abstract). Mitragotri discloses that delivery is enhanced using liposome or microparticle drug carriers, particularly with microparticles having surfaces with increased hydrophilicity or lipophilicity (abstract). There is no disclosure or suggestion in Mitragotri, however, of a pH-sensitive polymer which enhances transport through cell or cell component membranes by becoming more hydrophobic and more lipophilic at a pH between about 5 and 6.5 and which is conjugated to or has incorporated therein a second polymeric or monomeric unit which bonds to a carrier or a therapeutic or diagnostic agent.

Hoffman

Hoffman discloses stimuli-responsive polymers conjugated to interactive molecules. The function of the interactive molecule is controlled by a change in an external stimuli, such as temperature or pH, and the change cause the polymer to undergo a conformational or physico-chemical change which leads to a structural transition at or near or distant to the site of attachment, thereby modulating the activity of the interactive molecule in the process (p. 9, lines 9-23). There is no disclosure or suggestion in Hoffman, however, of a pH-sensitive polymer which enhances transport through a membrane at a pH between about 5 and 6.5 by becoming more hydrophobic and lipophilic *and which is conjugated to or has incorporated therein a second polymeric or monomeric unit which bonds to a carrier or a therapeutic or diagnostic agent.*

Shapland

Shapland discloses a drug delivery apparatus and method for delivering a drug encapsulated in a polymeric matrix to internal body tissue using a catheter device and

iontophoresis or phonophoresis (abstract). Shapland fails, however, to disclose or suggest a pH-sensitive polymer which becomes more hydrophobic and more lipophilic at a pH between about 5 and 6.5 to thereby enhance transport through a cell or cell component membrane and which is conjugated to or has incorporated therein a second polymeric or monomeric unit which bonds to a carrier or a therapeutic or diagnostic agent.

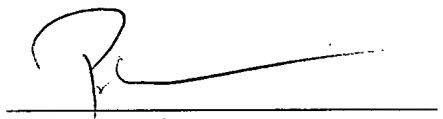
Lishko

Lishko discloses encapsulation of compounds in liposomes for targeted delivery to hair follicles (col. 3, lines 19-38). Lishko fails, however, to disclose or suggest a pH-sensitive polymer which becomes more hydrophobic and more lipophilic at a pH between about 5 and 6.5 and which is conjugated to or has incorporated therein a second polymeric or monomeric unit which bonds to a carrier or a therapeutic or diagnostic agent.

Lishko therefore fails to disclose the claimed conjugate or methods of use thereof. Lishko also does not make obvious that which is claimed since Lishko provides no teaching of enhancing transport through a cell wall or cell component membrane, nor for that matter, through a liposome. In fact, Lishko teaches away from such a use by teaching that one *should encapsulate compound within the liposomes, not that one should enhance the permeability of the liposomes*.

Applicants therefore respectfully request allowance of claims 1, 5, 7-13, 15, and 17-33, as amended.

Respectfully submitted,



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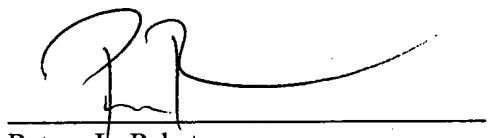

Patrea L. Pabst

Date: July 10, 2001

APPENDIX: Marked Copy of Claims as Amended

1. (Three times amended) A polymeric composition for enhancing transport through a cell membrane, cell component membrane or phospholipid membrane [membranes] comprising

a first pH-sensitive polymer which is [not] hydrophobic at a first pH, but which is more hydrophobic and more lipophilic and thereby enhances transport through [disrupts a] the cell membrane, cell component membrane, liposome or lipid vesicle at a second pH,

a second unit conjugated to, complexed with, or incorporated with the first pH- sensitive polymer, wherein the unit is selected from the group consisting of a carrier, a therapeutic agent, a diagnostic agent, and combinations thereof.

5. (three times amended) The composition of claim 1 [comprising] wherein the second unit comprises a therapeutic or diagnostic agent, the composition further comprising a pharmaceutically acceptable carrier.

7. (three times amended) The composition of claim 1 wherein the second unit comprises a polymer and the first polymer and the second unit form a graft copolymer, block copolymer, random copolymer or blend thereof.

8. (twice amended) The composition of claim 1 wherein the second unit is linked to a ligand binding to the surface of a cell.

9. The composition of claim 1 further comprising a compound which decreases lysosomal degradation.

10. The composition of claim 5 wherein the therapeutic agent is a cytotoxic compound.

11. (twice amended) The composition of claim 1 wherein the second unit comprises a polycationic polymer or cationic lipid.

12. (Once amended) The composition of claim 5 wherein the therapeutic agent is a nucleoside, nucleotide, or nucleic acid.

13. (Once amended) The composition of claim 1 further comprising a carrier selected from the group consisting of microparticles, nanoparticles, micelles and liposomes.

Please cancel claim 15.

17. (amended) The method of claim [15] 33 wherein the composition is administered to

cells in a suspension.

18. (amended) The method of claim [15] 33 wherein the composition is administered to layers of cells to enhance transport through the cell layers.

19. (amended) The method of claim [15] 33 wherein the composition is administered to [lipid membranes] liposomes or lipid vesicles to enhance transport of molecules into or out of the [lipid membranes] liposomes or lipid vesicles.

20. (three times amended) The method of claim [15] 33 wherein the composition is administered in combination with electrophoresis or iontophoresis.

21. (twice amended) The method of claim [15] 33 further comprising application of a stimulus means to further enhance the effectiveness of the composition to [disrupt] alter transport through the membrane, wherein the stimulus means induces a change in the structure of the polymer of the composition.

22. (twice amended) The method of claim 21 wherein the stimulus means is selected from the group consisting of changes in pH, light, ionic strength, solvent [composition] to alter solubility of the composition, temperature, and electric field.

23. (amended) The method of claim [15] 33 further comprising administration of a stimulus means to further enhance the effectiveness of the composition to [disrupt] alter transport through the membrane, wherein the stimulus means is selected from the group consisting of ultrasound, electrical fields, radiation, and combinations thereof.

24. The method of claim 23 wherein the stimulus means is ultrasound.

25. The method of claim 24 wherein the ultrasound is administered at between 20 kHz and 10 MHz.

26. (twice amended) The composition of claim 11 wherein the polycationic material is selected from the group consisting of chitosan, polylysine, polyethyleneimine, poly(propyleneimine), aminodextran, collagen, polyvinylimidazole, and N,N-dimethylaminoethyl methylacrylate.

27. The composition of claim 13 wherein the carrier is a micelle or liposome.

28. (twice amended) The composition of claim 7 wherein the pH sensitive polymer is

selected from the group consisting of acrylic acid polymers; C_{1-6} straight chain, branched, ethylene-acrylic acid copolymers and cyclic 2-alpha-alkyl acrylic acids; vinyl imidazole polymers and esters of acrylic acid copolymerized with acrylic acid.

29. (twice amended) The composition of claim 7 wherein the second [units comprise] unit comprises polymeric blocks comprising proteins or peptides which include imidazole groups.

30. (amended) The composition of claim 1 wherein the second unit comprises a lipid or phospholipid.

31. (amended) The composition of claim 1 wherein the second unit comprises sulfonated groups.

32. (amended) The composition of claim 1 wherein the second unit is sensitive to a stimulus selected from the group consisting of temperature, light, electrical stimuli, radiation, pH and ion concentration.

Please add new claim 33.

33. A method for enhancing transport of agents through cell membranes, cell component membranes or liposomes or lipid vesicles comprising administering to the cell membrane cell component membrane, liposome or lipid vesicle any of the compositions of claims 1, 5, 7-13, and 26-32.

Appendix: Clean Copy of Claims as Amended

D1

1. (Three times amended) A polymeric composition for enhancing transport through a cell membrane, cell component membrane or phospholipid membrane comprising

a first pH-sensitive polymer which is hydrophobic at a first pH, but which is more hydrophobic and more lipophilic and thereby enhances transport through the cell membrane, cell component membrane, liposome or lipid vesicle at a second pH,

a second unit conjugated to, complexed with, or incorporated with the first pH- sensitive polymer, wherein the unit is selected from the group consisting of a carrier, a therapeutic agent, a diagnostic agent, and combinations thereof.

D2

5. (three times amended) The composition of claim 1 wherein the second unit comprises a therapeutic or diagnostic agent, the composition further comprising a pharmaceutically acceptable carrier.

D3

7. (three times amended) The composition of claim 1 wherein the second unit comprises a polymer and the first polymer and the second unit form a graft copolymer, block copolymer, random copolymer or blend thereof.

8. (twice amended) The composition of claim 1 wherein the second unit is linked to a ligand binding to the surface of a cell.

9. The composition of claim 1 further comprising a compound which decreases lysosomal degradation.

10. The composition of claim 5 wherein the therapeutic agent is a cytotoxic compound.

D4

11. (twice amended) The composition of claim 1 wherein the second unit comprises a polycationic polymer or cationic lipid.

12. (Once amended) The composition of claim 5 wherein the therapeutic agent is a nucleoside, nucleotide, or nucleic acid.

13. (Once amended) The composition of claim 1 further comprising a carrier selected from the group consisting of microparticles, nanoparticles, micelles and liposomes.

D5

17. (amended) The method of claim 33 wherein the composition is administered to cells

D5 cont'd

in a suspension.

18. (amended) The method of claim 33 wherein the composition is administered to layers of cells to enhance transport through the cell layers.

19. (amended) The method of claim 33 wherein the composition is administered to liposomes or lipid vesicles to enhance transport of molecules into or out of the liposomes or lipid vesicles.

20. (three times amended) The method of claim 33 wherein the composition is administered in combination with electrophoresis or iontophoresis.

21. (twice amended) The method of claim 33 further comprising application of a stimulus means to further enhance the effectiveness of the composition to alter transport through the membrane, wherein the stimulus means induces a change in the structure of the polymer of the composition.

22. (twice amended) The method of claim 21 wherein the stimulus means is selected from the group consisting of changes in pH, light, ionic strength, solvent to alter solubility of the composition, temperature, and electric field.

23. (amended) The method of claim 33 further comprising administration of a stimulus means to further enhance the effectiveness of the composition to alter transport through the membrane, wherein the stimulus means is selected from the group consisting of ultrasound, electrical fields, radiation, and combinations thereof.

24. The method of claim 23 wherein the stimulus means is ultrasound.

25. The method of claim 24 wherein the ultrasound is administered at between 20 kHz and 10 MHz.

D6

26. (twice amended) The composition of claim 11 wherein the polycationic material is selected from the group consisting of chitosan, polylysine, polyethyleneimine, poly(propyleneimine), aminodextran, collagen, polyvinylimidazole, and N,N-dimethylaminoethyl methylacrylate.

27. The composition of claim 13 wherein the carrier is a micelle or liposome.

D7

28. (twice amended) The composition of claim 7 wherein the pH sensitive polymer is

D7 cont'd

selected from the group consisting of acrylic acid polymers; C_{1-6} straight chain, branched, ethylene-acrylic acid copolymers and cyclic 2-alpha-alkyl acrylic acids; vinyl imidazole polymers and esters of acrylic acid copolymerized with acrylic acid.

29. (twice amended) The composition of claim 7 wherein the second unit comprises polymeric blocks comprising proteins or peptides which include imidazole groups.

30. (amended) The composition of claim 1 wherein the second unit comprises a lipid or phospholipid.

31. (amended) The composition of claim 1 wherein the second unit comprises sulfonated groups.

32. (amended) The composition of claim 1 wherein the second unit is sensitive to a stimulus selected from the group consisting of temperature, light, electrical stimuli, radiation, pH and ion concentration.

D8

33. A method for enhancing transport of agents through cell membranes, cell component membranes or liposomes or lipid vesicles comprising administering to the cell membrane cell component membrane, liposome or lipid vesicle any of the compositions of claims 1, 5, 7-13, and 26-32.

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Molecular engineering of proteins and polymers for targeting and intracellular delivery of therapeutics.

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There are many protein and DNA based therapeutics under development in the biotechnology and pharmaceutical industries. Key delivery challenges remain before many of these biomolecular therapeutics reach the clinic. Two important barriers are the effective targeting of drugs to specific tissues and cells and the subsequent intracellular delivery to appropriate cellular compartments. In this review, we summarize protein engineering work aimed at improving the stability and refolding efficiency of antibody fragments used in targeting, and at constructing new streptavidin variants which may offer improved performance in pre-targeting delivery strategies. In addition, we review recent work with pH-responsive polymers that mimic the membrane disruptive properties of viruses and toxins. These polymers could serve as alternatives to fusogenic peptides in gene therapy formulations and to enhance the intracellular delivery of protein therapeutics that function in the cytoplasm.

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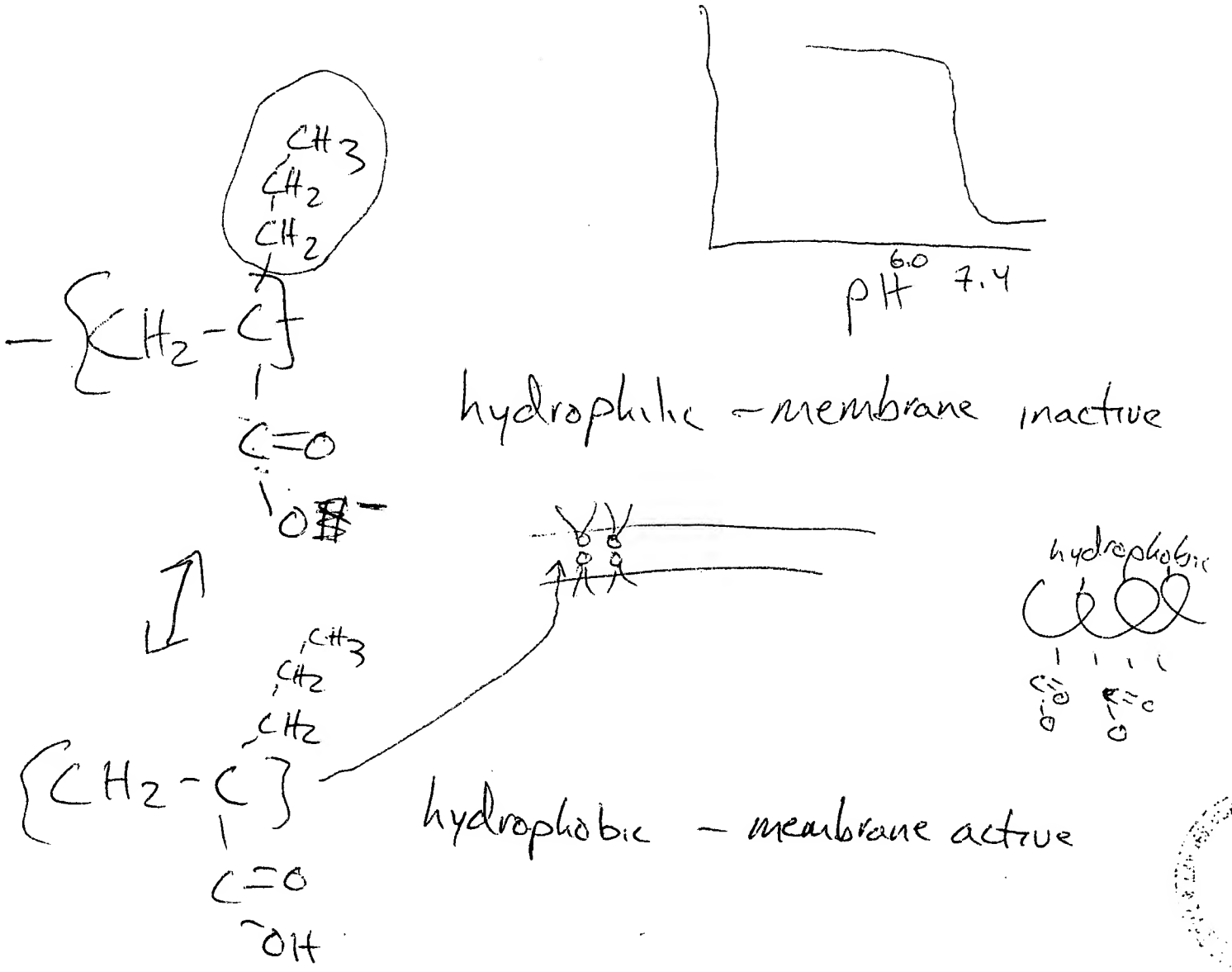
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hydrophobic
hydrophobic – membrane active